Exhibit 2

Pain 113 (2005) 271–276

PAIN

www.elsevier.com/locate/pain

Spinal noradrenaline transporter inhibition by reboxetine and Xen2174 reduces tactile hypersensitivity after surgery in rats

Hideaki Obata[a,b], Dawn Conklin[a], James C. Eisenach[a,*]

[a]*Department of Anesthesiology and Center for the Pharmacologic Plasticity in the Presence of Pain, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157*

[b]*Department of Anesthesiology, Gunma University Graduate School of Medicine, 3-39-22 Showa, Maebashi, Gunma 371-8511, Japan*

Received 6 May 2004; received in revised form 16 September 2004; accepted 18 October 2004

Abstract

Spinal noradrenaline (NA) released in response to noxious stimuli may play an important role in suppression of nociceptive transmission. Here, we investigated the efficacy of a competitive NA transporter inhibitor (reboxetine) and a noncompetitive NA transporter inhibitor peptide, Xen2174, isolated from the Pacific cone snail, to treat tactile hypersensitivity following paw incisional surgery. Male Sprague–Dawley rats were anesthetized, an incision of the plantar aspect of the hind paw was performed, and withdrawal threshold to von Frey filaments near the surgical site determined. Reboxetine (0.5–5 μg) and Xen2174 (0.3–100 μg) increased withdrawal threshold when injected 24 h after paw incision, with a peak effect at 15–60 min, for Xen2174, an ED50 value of 0.64 μg. Administration of Xen2174 (3–30 μg) 15 min before incision also reduced hypersensitivity in a dose-dependent manner. Withdrawal threshold after the single 30 μg dose was greater than vehicle control even at 2, 3, and 5 days after incision. Doses ≤30 μg did not alter spontaneous behavior. The anti-hypersensitivity effect of 10 μg of Xen2174 was totally blocked by the α2-adrenoceptor antagonist, idazoxan, and partially blocked by the muscarinic antagonist, atropine. These data suggest that selective NA transporter inhibition suppresses post-incisional hypersensitivity through a different mechanism from that of neuropathic pain, since we previously reported that reversal of hypersensitivity by intrathecal clonidine, an α2-adrenoceptor agonist, following spinal nerve ligation is completely blocked by intrathecal atropine. Finally, these data suggest that intrathecal administration of Xen2174 at the time of spinal anesthesia might produce postoperative analgesia in humans.

Keywords: Xen2174; Noradrenaline transporter; Postoperative pain; Intrathecal; Rats; Reboxetine

1. Introduction

Noradrenaline (NA) plays an important role in inhibition of nociceptive transmission in the spinal cord. NA-containing fibers descend from the A5, A6 and A7 cell loci in the pons to the spinal cord (Fritschy and Grzanna, 1990; Westlund et al., 1983), and electrical stimulation of these pontine loci results in analgesia (Jones and Gebhart, 1986; Yeomans et al., 1992). This noradrenergic inhibitory pathway is also activated by administration of opioids, electrical stimulation of the periaqueductal gray, or persistent noxious input (Aimone et al., 1987; Bajic and Proudfit, 1999; Camarata and Yaksh, 1985; Satoh and Omote, 1996; Tyce and Yaksh, 1981). Intrathecal administration of NA itself produces an antinociceptive effect to acute noxious heat such as the traditional tail-flick and hot plate tests in rats (Howe et al., 1983; Reddy et al., 1980).

The role of descending NA activity on persistent pain in the peri-operative period has not previously been examined. One goal of the current study was to probe the activity of this system by testing whether its amplification by intrathecal injection of NA transporter inhibitors would reduce tactile hypersensitivity in a rat model of post-operative pain (Brennan et al., 1996). For this purpose we utilized reboxetine, an antidepressant with selectivity for the NA transporter (Miller et al., 2002). In addition, we utilized a novel NA transporter inhibitor peptide, Xen2174, derived

* Corresponding author. Tel.: +1 336 716 4182; fax: +1 336 716 0288.
E-mail address: jim@eisenach.us (J.C. Eisenach).

0304-3959/$20.00
doi:10.1016/j.pain.2004.10.017

from the venom of the marine cone snail *Conus marmoreus* (Sharpe et al., 2001). Its principle mode of action is non-competitive inhibition, through allosteric modulation, of the NA transporter. Xen2174 differs from classically described inhibitors of the NA transporter such as antidepressants which act in a competitive fashion, resulting in inhibition of NA reuptake in the synaptic cleft (Bryan-Lluka et al., 2003; Sharpe et al., 2001; Sharpe et al., 2003), resulting in analgesia in mice after intrathecal injection (McIntosh et al., 2000). We also examined preemptive treatment with Xen2174, administered prior to surgery, since spinal injections are most commonly administered clinically just prior to surgery in order to establish spinal anesthesia with local anesthetic. We assumed that descending NA tone would be less active prior to and during surgery under general anesthesia than in the postoperative period, and hypothesized that Xen2174 would have a lesser effect when given before surgery than on the first postoperative day.

Previous studies indicate that spinal NA stimulates α1-adrenoceptors on inhibitory interneurons which contain γ-aminobutyric acid and glycine (Baba et al., 2000), and stimulates α2-adrenoceptors to reduce afferent release of glutamate (Kawasaki et al., 2003), resulting in antinociception. Intrathecal administration of α2-adrenoceptor agonists suppresses mechanical hypersensitivity after paw incision (Duflo et al., 2003). We have shown that spinal circuitry activated by α2-adrenoceptor agonists changes remarkably after peripheral nerve injury. Thus, the inhibitory effect of intrathecal clonidine in neuropathic pain is completely blocked by destruction of cholinergic interneurons (Paqueron et al., 2001) or by antagonism of muscarinic receptors (Pan et al., 1999). The relevance of these observations in chronic nerve injury models to postoperative analgesia mechanisms from clonidine are unknown. Thus, a final purpose was to evaluate whether the effect of Xen2174 could be blocked by α2-adrenergic or muscarinic antagonists in this postoperative pain model.

2. Methods

2.1. Surgical preparation

The study was approved by the Animal Care and Use Committee of Wake Forest University School of Medicine (Winston-Salem, North Carolina). Male Sprague–Dawley rats (250 g) obtained from Harlan (Indianapolis, IN) were used in all experiments. Animals were housed under a 12-h light-dark cycle, with food and water *ad libitum*. For intrathecal administration, a sterilized 32-gauge polyethylene catheter (RecathCo, Allison Park, PA) connected to an 8.5 cm Tygon external tubing (Saint–Gobain Performance Plastics, Akron, OH) was inserted under halothane anesthesia, as previously described (Yaksh and Rudy, 1976). The catheter was passed caudally 7.5 cm from the cisterna magnum to the lumbar enlargement. Only animals without evidence of neurologic dysfunction after catheter insertion were used for studies. Paw incision as previously described (Brennan et al., 1996) was performed 5 days after intrathecal catheter implantation. For this, rats were anesthetized with halothane, and after sterile preparation with 70% ethanol, a 1 cm long incision was made in the plantar aspect of the left hind paw, starting 0.5 cm from edge of the heel toward the toe. The plantaris muscles was elevated and incised longitudinally. The wound was closed with 2 mattress sutures of 5.0 silk.

2.2. Behavioral testing

Rats were placed individually in a plastic cage with a plastic mesh floor, and allowed to acclimate to the environment for 30 min. Withdrawal threshold was determined using calibrated von Frey filaments (Stoelting, Wood Dale, IL), beginning with the 2.0-gauge filament. Filaments were applied vertically to an area adjacent to the wound for 6 s while the filament was gently bent. In the absence of a response, the filament of next greater force was applied. In the presence of a response, the filament of next lower force was applied. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the up-down method, as previously described (Chaplan et al., 1994).

Changes in general behavior, including vocalization, repetitive movements, and activity level, were noted throughout the time of testing.

2.3. Drugs and their administration

All studies were performed in a randomized, blinded manner. Intrathecal reboxetine and Xen2174 alone or with antagonists were performed 24 h after paw incision. The withdrawal threshold was determined before (prepaw incision threshold) and 24 h after incision (baseline), then at 15 min, 30 min, and every 30 min for 4 h after intrathecal injection. Rats received intrathecal reboxetine (0.5, 5 μg), Xen2174 (0.3, 1, 3, 10 and 100 μg) or vehicle with 5–7 animals per group. Doses of reboxetine were determined from preliminary experiments in which 0.05 μg was without effect and doses >5 μg had no greater effect than 5 μg. For antagonist studies, rats received an intrathecal injection of saline, the selective α2-adrenoceptor antagonist, idazoxan, 30 μg, or the selective muscarinic receptor antagonist, atropine, 30 μg with 6 animals per group. Fifteen minutes later all animals received Xen2174, 10 μg intrathecally. Doses of antagonists were chosen based on work by us to block their respective receptors after intrathecal administration (Li et al., 2002; Pan et al., 1999).

In studies with preemptive injection, after determining paw withdrawal threshold (prepaw incision threshold), rats received intrathecal Xen2174 (3, 10 and 30 μg) or vehicle 15 min prior to paw incision, with 6 animals per group. Paw withdrawal thresholds were determined 1 h after incision, then every hour for 5 h, and on postoperative day 1, 2, 3 and 5 after incision. Drugs were administered intrathecally in a volume of 5 μl followed by a 10 μl of saline injection to flush the catheter. Xen2174 was obtained from Xenome Ltd (Indooroopilly, Australia). Idazoxan and atropine were purchased from Sigma Chemical Co. (St. Louis, MO). Xen2174 was dissolved in 5 mM of sodium acetate in saline. Idazoxan and atropine were dissolved in saline.

2.4. Statistics

Data were normally distributed and are shown as mean ± SEM of withdrawal thresholds or percentages of maximum possible effect (%MPE), which was calculated as 100× (postdrug response-baseline)/(prepaw incision threshold-baseline). Dose-response curves were constructed at the time of peak effect at each dose, and were analyzed by fitting to a Boltzmann sigmoidal dose response using Origin (OriginLab, Northampton, MA). Data were analyzed using a two-way analysis of variance (ANOVA), followed by a Student-Newman-Keuls post-hoc test. A P value of less then 0.05 was considered to indicate statistical significance.

3. Results

3.1. Dose response postoperative study

Xen2174 produced a dose-dependent anti-hypersensitivity effect with a peak effect 15 min after injection and duration of 30–180 min, depending on the dose ($P<0.05$ by two-way ANOVA, Fig. 1). Total reversal of postoperative hypersensitivity was observed with the 10 μg dose. Increasing the dose to 100 μg did not increase the peak effect or duration, but resulted in hyper locomotion without vocalization and serpentine tail movements in all animals, lasting <60 min. Other doses were without effects on spontaneous behavior. Vehicle treatment did not alter withdrawal thresholds. The ED50 for Xen2174 was 0.64 μg ($r=0.95$), with a threshold effect of 0.3 μg and a plateau maximum effect at 10 μg (Fig. 2). Reboxetine also increased withdrawal threshold after intrathecal injection, with a peak effect 60 min after injection. In pilot experiments, doses of reboxetine >5 μg resulted in urination, and no greater effect on withdrawal threshold than 5 μg. Peak effect of reboxetine was 34 ± 14%MPE after 5 μg (Fig. 2; $P<0.05$ compared to vehicle control).

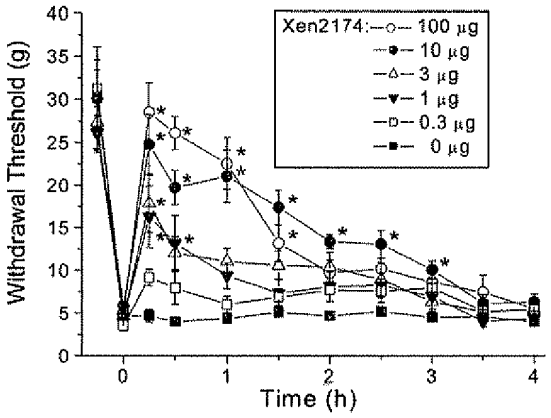

Fig. 1. Time course of the anti-hypersensitivity effects of intrathecally administered Xen2174 in rats 1 day after paw incision surgery. Presurgery baseline values are indicated at the time point before 0. Withdrawal thresholds are expressed at mean ± SEM for six or seven rats in each group. *$P<0.05$ versus vehicle-treated group.

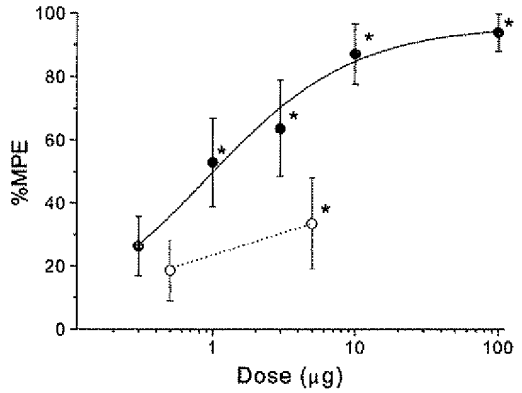

Fig. 2. Log dose-response curve of intrathecal administration of reboxetine (open circles) or Xen2174 (closed circles) on paw incision-induced mechanical hypersensitivity. Peak effects were used to calculate percentages of the maximum possible effects (%MPE). Data are expressed as mean ± SEM for five to seven rats in each group. * $P<0.05$ compared to vehicle control.

3.2. Preemptive treatment study

Intrathecal injection of Xen2174 before incision produced a dose-dependent anti-hypersensitivity effect ($P<0.05$ by two-way ANOVA, Fig. 3). Withdrawal thresholds after 30 μg were greater than vehicle control even at 2, 3, and 5 days after incision. The anti-hypersensitivity effects of 10 μg administered preoperatively lasted 5 h, which was longer than the effect when the same dose was injected in rats 24 h after incision (3 h duration). Intrathecal injection of 30 μg of Xen2174 did not alter spontaneous behavior.

3.3. Antagonist study

Xen2174, 10 μg, increased withdrawal threshold to over 90% return to pre-surgery values, similar to the dose-response study described above. This reduction of

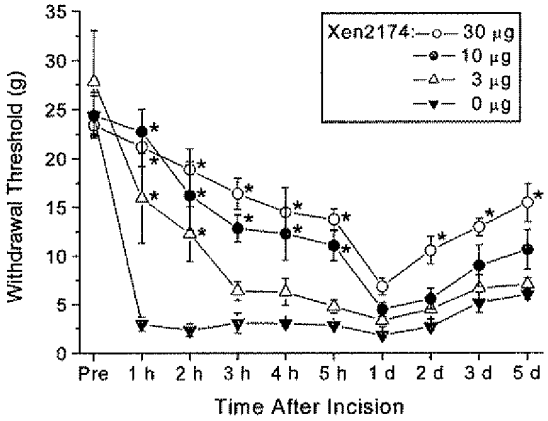

Fig. 3. The anti-hypersensitivity effects of preemptive intrathecal administration of Xen2174 in rats with paw incision surgery. Xen2174 was injected 15 min before paw incision. Withdrawal thresholds are expressed at mean ± SEM for six rats in each group. *$P<0.05$ versus vehicle-treated group.

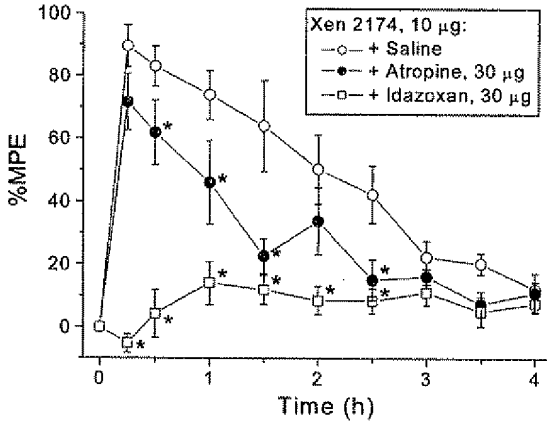

Fig. 4. Effects of intrathecal pretreatment of idazoxan, an α2-adrenoceptor antagonists, or atropine, a muscarinic receptor antagonist, on the anti-hypersensitivity effects of 10 μg of Xen2174. Rats were pretreated with saline, idazoxan, or atropine 15 min before Xen2174 administration. Data are expressed as mean ± SEM for six rats in each group. *$P < 0.05$ versus the group treated with saline plus Xen2174. %MPE = percentage of the maximum possible effect.

postoperative hypersensitivity by Xen2174 was totally blocked by the α2-adrenoceptor antagonist idazoxan, and partially blocked by the muscarinic antagonist atropine ($P < 0.05$ by two-way ANOVA, Fig. 4). Intrathecal administration of idazoxan and atropine alone at these doses produced slight agitation, but did not alter withdrawal threshold, which was 5.2 ± 0.4 g before idazoxan and a nadir of 4.6 ± 0.5 g after idazoxan, or 3.9 ± 0.3 g before atropine and a nadir of 4.6 ± 0.5 g after atropine.

4. Discussion

In the present study, intrathecal administration of chemical (reboxetine) and peptidergic (Xen2174) selective NA transporter inhibitors, reduced postoperative hypersensitivity in a validated model of incisional pain and tactile hypersensitivity. Xen2174 was more efficacious in this regard, capable of producing complete reversal of hypersensitivity at doses without overt effects on behavior. Although the administration of 100 μg Xen2174 produced hyper locomotion and serpentile tail movements, this dose was 10 times the just maximally effective dose against hypersensitivity. This is in contrast to our previous experience with the direct noradrenergic agonist, clonidine, in this model, which demonstrated a maximum efficacy of only 35% of return to pre-surgery sensitivity, and only at doses which produced marked sedation and diuresis (Duflo et al., 2003). Clonidine also reduces blood pressure when administered neuraxially in humans (Mendez et al., 1990), and we cannot comment on this potential adverse event, since invasive blood pressure was not measured in this study. Reboxetine was less efficacious than Xen2174, perhaps reflecting its antagonism of nicotinic cholinergic receptors of subtypes thought to be antinociceptive at spinal sites (Miller et al., 2002).

4.1. Activity of descending NA inhibition after surgery

There is no evidence that NA transporter inhibition in the central nervous system produces antinociception in the normal animal (Jasmin et al., 2003), and genetically modified mice lacking the NA transporter exhibit normal nociceptive threshold (Bohn et al., 2000). In contrast, persistent nociception activates descending NA inhibition, as indicated by increases in NA in lumbar intrathecal space perfusates (Tyce and Yaksh, 1981), and suppression by intrathecal administration of a NA transporter preferring inhibitor, desipramine, of inflammatory pain (Kawamata et al., 1999). The current study is the first to demonstrate that chemical and peptidergic NA transporter inhibitors reduce hypersensitivity in a model of postoperative pain.

These observations suggest that descending noradrenergic inhibitory systems are activated in the postoperative condition and that inhibition of NA reuptake can suppress tactile hypersensitivity following incisional surgery, at doses without side effects observed with direct NA agonists. If such descending NA inhibition is active, one might have expected increasing hypersensitivity (further reduced withdrawal threshold) following intrathecal injection of the α2-adrenoceptor antagonist, idazoxan, alone. This was not observed, perhaps due to our inability, using the standard von Frey filaments, to observe yet more hypersensitivity in these animals rendered hypersensitive after surgery. We recognize also that selectivity of all pharmacologic probes is reduced in a dose dependent fashion, and it is conceivable that the anti-hypersensitivity effects of reboxetine and Xen2174 were due to other actions than at the NA transporter. However, their only known common action is at this site, and inhibition of Xen2174's effect by idazoxan strongly favors a NA transporter mechanism.

Of course, spontaneous, ongoing pain is not easily assessed in this model, so the degree of analgesia which might be experienced by patients is unknown. However, manipulations which reduce tactile hypersensitivity in patients after surgery also produce analgesia (Wilder-Smith et al., 2003), suggesting that this treatment would be effective in humans.

4.2. Preemptive treatment with NA transporter inhibitors

The results of preemptive treatment with Xen2174, administered prior to surgery were somewhat surprising. Our hypothesis was that descending noradrenergic tone would be less active prior to and during surgery with general anesthesia than in the postoperative period, and we expected that pre-incisional injection of Xen2174 would have a lesser effect than when it was administered one day after incision. This was not observed. Not only was preoperative administration of 10 μg of Xen2174 effective longer than the effect

when injected one day after incision, but 30 μg of Xen2174 administered preoperatively had a lingering effect that lasted for 5 days during recovery. This result suggests that descending NA inhibitory systems are already activated even during and in the early period after surgery. Our result also suggests that single pre-operative administration, such as a part of a spinal anesthetic, could result in intense analgesia for several hours and improvement in pain relief for several days. We note that increased efficacy of drugs before surgery rather than 24 h later is not a generalized feature of this model, since it is not observed with intrathecal morphine (Brennan et al., 1997; Zahn et al., 1997).

Preemptive analgesia has been defined as 'antinociceptive treatment given before incision is more effective than that given after' (Kissin, 1996) and results from the current study suggest that Xen2174 is a preemptive analgesic. The long-lasting effects of preemptive intrathecal Xen2174 may also be important for a practical reason. Epinephrine and phenylephrine are often used in spinal anesthesia together with local anesthetics in order to decrease the clearance of the local anesthetics from the subarachniod space (Conception et al., 1984; Vaida et al., 1986). However, direct intrathecal injection of catecholamines may induce ischemia of the spinal cord by vasoconstriction of the spinal arterial supply (Tetzlaff et al., 1998). Coadministration with an agent such as an NA transporter inhibitor, which increases the NA concentrations primarily at sites of neuronal interaction, might be a promising way to prolong the effect of intrathecally administered local anesthetics for spinal anesthesia without this risk.

4.3. Spinal NA mechanisms of analgesia after surgery

Intrathecal injection of α2-adrenoceptor agonists diminishes tactile hypersensitivity following paw incision (Duflo et al., 2003). The antagonist study in the current study is consistent with these observations, demonstrating clearly that Xen2174 works primarily by a noradrenergic mechanism, since it is totally reversed by the α2-adreno-adrenoceptor antagonist, idazoxan. Stimulation of spinal cholinergic systems by intrathecal injection of α2-adrenoceptor agonists is widely documented in humans and animals (Eisenach, 1999). For example, intrathecal, but not intravenous clonidine increases acetylcholine concentrations in cerebrospinal fluid (de Kock et al., 1997). The reliance of antinociception from clonidine on this spinal cholinergic interaction varies between normal and nerve-injured animals. Intrathecal clonidine antinociception to acute thermal stimuli in normal rats is unaffected by intrathecal atropine (Paqueron et al., 2003), but the reversal of hypersensitivity by clonidine following spinal nerve ligation is completely blocked by intrathecal atropine (Pan et al., 1999).

The emerging literature suggests that postoperative pain exhibits a unique pharmacology of analgesia compared with other sustained pain models. For example, although spinal *N*-methyl-D-aspartate (NMDA) receptor antagonists attenuate hypersensitivity in most models of persistent pain, these are not effective to treat hypersensitivity after surgical incision (Zahn and Brennan, 1998a). In contrast, intrathecal administration of non-NMDA receptor antagonists (Zahn et al., 1998b), NK-1 receptor antagonists (Yamamoto and Sakashita, 1999) and cyclooxygenase-1 inhibitors are effective (Zhu et al., 2003). These observations suggest that mechanisms of hypersensitivity after incision differ those following inflammatory or peripheral nerve injury. We speculate that the muscarinic dependency of spinal α2-adrenoceptor mediated analgesia increases progressively from acute nociception in normal animals to postoperative hypersensitivity to nerve injury induced chronic hypersensitivity. The relatively modest reduction by atropine in the response to Xen2174 one day after surgery in the current study supports this hypothesis, and suggests that there is a small dependence on a cholinergic circuit for NA-mediated analgesia in this setting.

In summary, intrathecal administration of reboxetine and Xen2174 reduces hypersensitivity in a rat model of postoperative pain without adverse behavioral effects at their just maximum effective doses. Preemptive intrathecal administration of Xen2174 also suppresses post-incisional hypersensitivity in a dose-dependent manner and this effect lasts several days after surgery. Partial reversal only of Xen2174 by intrathecal atropine suggests that the nature of postoperative pain is different from that of persistent pain states following nerve injury. These data suggest that selective NA reuptake inhibition in the spinal cord represents a promising approach to inhibit postoperative hypersensitivity.

Acknowledgements

This work was supported in part by a grant from Xenome Ltd and NIH grant NS41386.

References

Aimone LD, Jones SL, Gebhart GF. Stimulation-produced descending inhibition from the periaqueductal gray and nucleus raphe magnus in the rat: mediation by spinal monoamines but not opioids. Pain 1987;31:123–36.

Baba H, Shimoji K, Yoshimura M. Norepinephrine facilitates inhibitory transmission in substantia gelatinoisa of adult rat spinal cord (part I): effects on axon terminals of GABAregic and glycinergic neurons. Anesthesiology 2000;92:473–84.

Bajic D, Proudfit HK. Projections of neurons in the periaqueductal gray to pontine and medullary catecholamine cell groups involved in the modulation of nociception. J. Comp. Neurol. 1999;405:359–79.

Bohn LM, Xu F, Gainetdinov RR, Caron MG. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. J Neurosci 2000;20:9040–5.

Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. Pain 1996;64:493–501.

Brennan TJ, Umali EF, Zahn PK. Comparison of pre- versus post-incision administration of intrathecal bupivacaine and intrathecal morphine in a rat model of postoperative pain. Anesthesiology 1997;87:1517–28.

Bryan-Lluka LJ, Bönisch H, Lewis RJ. χ-Conopeptide MrIA partially overlaps desipramine and cocaine binding sites on the human norepinephrine transporter. J Biol Chem 2003;278:40324–9.

Camarata PJ, Yaksh TL. Characterization of the spinal adrenergic receptors mediating the spinal effects produced by the microinjection of morphine into the periaqueductal gray. Brain Res 1985;336:133–42.

Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh YL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Meth 1994;53: 55–63.

Conception M, Maddi R, Francis D, Rocco AG, Murray E, Covino BG. Vasoconstrictors in spinal anesthesia with tetracaine - a comparison of epinephrine and phenylephrine. Anesth Analg 1984;63:134–8.

De Kock M, Eisenach J, Tong C, Schmitz AL, Scholtes JL. Analgesic doses of intrathecal but not intravenous clonidine increase acetylcholine in cerebrospinal fluid in humans. Anesth Analg 1997;84:800–3.

Duflo F, Conklin D, Li X, Eisenach JC. Spinal adrenergic and cholinergic receptor interactions activated by clonidine in postincisional pain. Anesthesiology 2003;98:1237–42.

Eisenach JC. Muscarinic-mediated analgesia. Life Sci 1999;64:549–54.

Fritschy JM, Grzanna R. Demonstration of two separate descending noradrenergic pathways to the rat spinal cord: evidence for an intragriseal trajectory of locus coeruleus axons in the superficial layers of the dorsal horn. J Comp Neurol 1990;291:553–82.

Howe JR, Wang JY, Yaksh TL. Selective antagonism of the antinociceptive effect of intrathecally applied alpha adrenergic agonists by intrathecal prazosin and intrathecal yohimbine. J Pharmacol Exp Ther 1983;224: 552–8.

Jasmin L, Tien D, Janni G, Ohara PT. Is noradrenaline a significant factor in the analgesic effect of antidepressants? Pain 2003;106:3–8.

Jones SL, Gebhart GF. Quantitative characterization of ceruleospinal inhibition of nociceptive transmission in the rat. J Neurophysiol 1986; 56:1397–410.

Kawamata T, Omote K, Kawamata M, Namiki A. Analgesic effect of intrathecal desipramine on carrageenan-induced thermal hyperalgesia in the rat. Br J Anaesth 1999;83:449–52.

Kawasaki Y, Kumamoto E, Furue H, Yoshimura M. α2 adrenoceptor-mediated presynaptic inhibition of primary afferent glutamatergic transmission in rat substantia gelatinosa neurons. Anesthesiology 2003; 98:682–9.

Kissin I. Preemptive analgesia: why its effect is not always obvious. Anesthesiology 1996;84:1015–9.

Li X, Conklin D, Ma W, Zhu X, Eisenach JC. Spinal noradrenergic activation mediates allodynia reduction from an allosteric adenosine modulator in a rat model of neuropathic pain. Pain 2002;97:117–25.

McIntosh JM, Corpuz GO, Layer RT, Garrett JE, Wagstaff JD, Bulaj G, Vyazovkina A, Yoshikami D, Cruz LJ, Olivera BM. Isolation and characterization of a novel conus peptide with apparent antinociceptive activity. J Biol Chem 2000;275:32391–7.

Mendez R, Eisenach JC, Kashtan K. Epidural clonidine analgesia after cesarean section. Anesthesiology 1990;73:848–52.

Miller DK, Wong EH, Chesnut MD, Dwoskin LP. Reboxetine: functional inhibition of monoamine transporters and nicotinic acetylcholine receptors. J Pharmacol Exp Ther 2002;302:687–95.

Pan HL, Chen SR, Eisenach JC. Intrathecal clonidine alleviates allodynia in neuropathic rats: interaction with spinal muscarinic and nicotinic receptors. Anesthesiolgy 1999;90:509–14.

Paqueron X, Li X, Bantel C, Tobin JR, Voytko ML, Eisenach JC. An obligatory role for spinal cholinergic neurons in the antiallodynic effects of clonidine after peripheral nerve injury. Anesthesiology 2001; 94:1074–81.

Paqueron X, Conklin D, Eisenach JC. Plasticity in action of intrathecal clonidine to mechanical but not thermal nociception after peripheral nerve injury. Anesthesiology 2003;99:199–204.

Reddy SVR, Maderdrut JL, Yaksh TL. Spinal cord pharmacology of adrenergic agonist-mediated antinociception. J Pharmacol Exp Ther 1980;213:525–33.

Satoh O, Omote K. Roles of monoaminergic, glycinergic and GABAergic inhibitory systems in the spinal cord in rats with peripheral mononeuropathy. Brain Res 1996;728:27–36.

Sharpe IA, Gehrmann J, Loughnan ML, Thomas L, Adams DA, Atkins A, Palant E, Craik DJ, Adams DJ, Alewood PF, Lewis RJ. Two new classes of conopeptides inhibit the α1-adrenoceptor and noradrenaline transporter. Nat Neurosci 2001;4:902–7.

Sharpe IA, Palant E, Schroeder CI, Kaye DM, Adams DJ, Alewood PF, Lewis RJ. Inhibition of the norepinephrine transporter by the venom peptide χ-MrIA. J Biol Chem 2003;278:40317–23.

Tetzlaff JE, Dilger J, Yap E, Smith MP, Schoenwald PK. Cauda equina syndrome after spinal anaesthesia in a patient with severe vascular disease. Can J Anaesth 1998;45:667–9.

Tyce GM, Yaksh TL. Monoamine release from cat spinal cord by somatic stimuli: An intrinsic modulatory system. J Physiol 1981; 314:513–29.

Vaida GT, Moss P, Capan LM, Turndorf H. Prolongation of lidocaine spinal anesthesia with phenylephrine. Anesth Analg 1986;65:781–5.

Westlund KN, Bowker RM, Ziegler MG, Coulter JD. Noradrenergic projections to the spinal cord of the rat. Brain Res 1983;263:15–31.

Wilder-Smith CH, Hill L, Dyer RA, Torr G, Coetzee E. Postoperative sensitization and pain after cesarean delivery and the effects of single IM doses of tramadol and diclofenac alone and in combination. Anesth Analg 2003;97:526–33.

Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.

Yamamoto T, Sakashita Y. The role of the spinal opioid receptor like1 receptor, the NK-1 receptor, and cyclooxygenase-2 in maintaining postoperative pain in the rat. Anesth Analg 1999;89:1203–8.

Yeomans DC, Clark FM, Paice JA, Proudfit HK. Antinociception induced by electrical stimulation of spinally projecting noradrenergic neurons in the A7 catecholamine cell group of the rat. Pain 1992;48:449–61.

Zahn PK, Gysbers D, Brennan TJ. Effect of systemic and intrathecal morphine in a rat model of postoperative pain. Anesthesiology 1997;86: 1066–77.

Zahn PK, Brennan TJ. Lack of effect of intrathecally administered *N*-methyl-D-aspartate receptor antagonists in a rat model for postoperative pain. Anesthesiology 1998a;88:143–56.

Zahn PK, Umali E, Brennan TJ. Intrathecal non-NMDA excitatory amino acid receptor antagonists inhibit pain behaviors in a rat model of postoperative pain. Pain 1998b;74:213–23.

Zhu X, Conklin D, Eisenach JC. Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. Pain 2003;104:15–23.